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(54) Title: RECEPTOR FOR *B. ANTHRACIS* TOXIN

(57) Abstract: The present invention relates to mammalian anthrax toxin receptor polypeptides and polynucleotides encoding same as well as related polypeptides and polynucleotides, vectors containing the polynucleotides and polypeptides, host cells containing related polynucleotide molecules, and cells displaying no anthrax toxin receptor on an exterior surface of the cells-minus cell lines and animals. The present invention also relates to methods for identifying molecules that bind the anthrax toxin receptor and molecules that reduce the toxicity of anthrax toxin. Finally, the present invention provides methods for treating human and non-human animals suffering from anthrax.

RECEPTOR FOR B. ANTHRACIS TOXIN

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application serial number 60/251,481, filed on December 5, 2000, which is incorporated herein by reference as if set forth in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH
OR DEVELOPMENT

[0002] Not applicable.

BACKGROUND OF THE INVENTION

[0003] *Bacillus anthracis*, the spore-forming causative agent of anthrax, generally infects herbivores (Hanna, 1998). Human infection, while rare, can result in a generally benign, self-limiting cutaneous disease or a systemic disease that rapidly leads to death in a high percentage of cases. The cutaneous disease can arise when spore particles from soil or animal products are introduced into cuts or skin abrasions. In contrast, the systemic disease can arise when *B. anthracis* spore particles are inhaled ($LD_{50} \approx 10,000$ spore particles). The high mortality rate and the ability to readily prepare and deliver *B. anthracis* spore particles as an aerosol have made *B. anthracis* a dreaded agent of biowarfare and bioterrorism.

[0004] The causative agent of the systemic disease is anthrax toxin (AT), which itself comprises a pair of binary, AB-type toxins – lethal toxin and edema toxin (Leppla, 1995). Each is assembled at the surface of mammalian cells from proteins released by *B. anthracis*. Lethal toxin, assembled from Protective Antigen (PA, 83 kDa) and Lethal Factor (LF, 90 kDa), is primarily responsible for lethality (Friedlander, 1986; Hanna et al., 1992; Hanna et al., 1993). Edema toxin, assembled from PA and Edema Factor (EF, 89 kDa), causes edema at the site of injection (Leppla, 1982). EF has calmodulin-dependent adenylate cyclase activity. LF is a Zn^{++} -dependent protease that cleaves certain proteins involved in signal transduction and cell cycle progression (MAPKK1 and MAPKK2) (Duesbery et al., 1998).

[0005] In these AB-type toxins, PA is the receptor-binding B moiety that delivers either EF or LF, as alternative enzymic A moieties, to the cytosol of mammalian cells (Leppla, 1995). Initially, PA binds specifically, reversibly, and with high affinity ($K_d \approx 1$ nM) to a cell-surface AT receptor (ATR). After binding to the receptor, PA is cleaved by a member of the furin family of proprotein convertases, which removes a 20 kDa fragment, PA20, from the N-terminus (Klimpel et al., 1992; Novak et al., 1992). The complementary fragment, PA63, remains receptor-bound and spontaneously self-associates to form heptameric ring-shaped oligomers (Milne et al., 1994) that avidly and competitively bind EF and/or LF (Leppla, 1995) to form EF/LF-PA63 complexes. These complexes are trafficked to an acidic compartment by receptor-mediated endocytosis. In the acidic compartment, the PA63 heptamers (the "prepore") are inserted into the membrane, forming transmembrane pores (Gordon et al., 1988). Concomitantly EF and LF are translocated across the membrane to the cytosol. Consistent with the pH dependence of translocation, toxin action is inhibited by lysosomotropic agents and baflomycin A1 (Mendard et al., 1996).

[0006] EF translocation causes a large increase in intracellular cAMP concentration (Gordon et al., 1988; Gordon et al., 1989). Increased cAMP levels cause edema, and in neutrophils, inhibit phagocytosis and oxidative burst (O'Brien et al., 1985). By protecting the bacteria from phagocytosis, edema toxin apparently aids in establishing bacterial infection and proliferation in the mammalian host.

[0007] Treatment of primary macrophages and certain macrophage cell lines with lethal toxin causes cell lysis (Friedlander, 1986). Macrophage-depleted mice are resistant to treatment with lethal toxin, suggesting that macrophages are the primary targets of lethal toxin (Hanna et al., 1993). Low doses of lethal toxin induce the production of interleukin-1 and tumor necrosis factor (Hanna et al., 1993). Thus, it has been suggested that hyperproduction of cytokines causes death of the host by inducing systemic shock. How these or other proteins lead to cytokine production and macrophage lysis remains unclear.

[0008] In the past few years considerable progress has been made toward a detailed understanding of the structure and function of PA. Crystallographic structures of PA and the PA63 heptamers have been determined (Petosa et al., 1997). The prepore undergoes a major conformational change under acidic conditions to form a 14-strand transmembrane β -barrel pore

(Benson et al., 1998; Miller et al., 1999). The pore structure and the detailed mechanism by which LF and EF are translocated across membranes are under intensive investigation.

[0009] The ATR structure is heretofore unknown, but is present in all cell lines that have been tested. Studies on CHO-K1 cells had indicated that PA binds to a proteinaceous receptor that is present in about 10^4 copies/cell (Escuyer and Collier, 1991). The paucity of knowledge about the ATR represents a major gap in the understanding of how AT acts. Identification and cloning of the ATR will provide more treatment strategies for anthrax.

[00010] A cDNA clone (Genbank Accession Number NM 032208) known as tumor endothelial marker 8 (TEM8) is known (St. Croix, 2000). TEM8 is upregulated in colorectal cancer endothelium, but heretofore the function of TEM8 was not known.

BRIEF SUMMARY OF THE INVENTION

[00011] The present application discloses structures of complete and partial anthrax toxin receptors from a mammal, namely a human. The complete anthrax toxin receptor includes an extracellular domain, a transmembrane domain, and a cytoplasmic domain that can vary in length, as is disclosed herein. It is disclosed herein that PA binds to the anthrax toxin receptor at a von Willebrand factor A (VWA) domain in the extracellular domain.

[00012] In one aspect, the invention is summarized in that an anthrax toxin receptor is a polypeptide having an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO: 8, SEQ ID NO:10, a PA-binding fragment of any of the foregoing, and a PA-binding variant of any of the foregoing polypeptides having conservative or non-conservative amino acid substitutions or other changes relative to the disclosed sequences. The various forms of the receptor encoded by SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO: 8, and SEQ ID NO:10 apparently differ as a result of alternative splicing.

[00013] In a related aspect, the invention further relates to an isolated polynucleotide that encodes any of the above-mentioned polypeptides and their complements, and a polynucleotide that hybridizes under moderately stringent or stringent hybridization conditions to any of the foregoing.

[00014] In still another related aspect, the invention encompasses a cloning vector and an expression vector comprising any of the foregoing polynucleotides, whether or not the

polynucleotide is operably linked to an expression control sequence that does not natively promote transcription or translation of the polynucleotide.

By identifying the polypeptides and polynucleotides of the invention, the applicant enables the skilled artisan to detect and quantify mRNA and ATR protein in a sample, and to generate *atr* transgenic and *atr* knock-out animals using methods available to the art.

[00015] Further, the invention includes a host cell comprising any such vector in its interior. Also within the scope of the present invention is a host cell having a polynucleotide of the invention integrated into the host cell genome at a location that is not the native location of the polynucleotide.

[00016] In yet another aspect, the invention is a method for producing an anthrax toxin receptor polypeptide that includes the steps of transcribing a polynucleotide that encodes an anthrax toxin receptor polypeptide, operably linked to an upstream expression control sequence, to produce an mRNA for the receptor polypeptide, and translating the mRNA to produce the receptor polypeptide. This method can be performed in a host cell when the polynucleotide is operably linked to the expression control sequence in an expression vector, and wherein the expression vector is delivered into a host cell, the expression control sequence being operable in the host cell. Alternatively, at least one of the transcribing and translating steps can be performed in an *in vitro* system, examples of which are well known in the art and commercially available. In either case, the polypeptide can be isolated from other cellular material using readily available methods.

[00017] In still another aspect, the invention is a method for identifying an agent that can alter the effect of AT on the host cell or organism. The method includes the steps of separately exposing a plurality of putative agents in the presence of AT to a plurality of cells having on their surface at least a portion of the ATR that binds to AT or a component thereof, comparing the effect of AT on the cells in the presence and absence of the agent, and identifying at least one agent that alters an effect of AT on the cells. In a related aspect, the present invention encompasses an agent that alters binding of AT to the ATR.

[00018] The present invention also encompasses a method for reducing or preventing AT-related damage *in vivo* or *in vitro* to human or non-human cells having an ATR on an outer cell

surface, the method comprising the step of exposing the cells to an agent that reduces binding of AT to the ATR. Similarly, the invention relates to a method for reducing or preventing damage *in vivo* or *in vitro* to human or non-human cells caused by AT by exposing AT to an agent that reduces binding of the AT to the ATR.

[00019] The present invention is also a method for identifying a mutant of the extracellular ATR domain or fragment thereof having altered (increased or reduced) binding affinity for AT.

[00020] It is an object of the invention to identify polypeptides that encode a mammalian anthrax toxin receptor, as well as fragments, mutants, and variants thereof and polynucleotides encoding same.

[00021] It is a feature of the invention that a soluble PA-binding polypeptide can reduce or eliminate toxicity associated with anthrax toxin.

[00022] Other objects, advantages and features of the invention will become apparent from the following specifications and claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[00023] Fig. 1 shows sequence alignment of various ATR polypeptide sequences with the I domain of integrin $\alpha 2$ and with the von Willebrand factor A domain consensus sequence.

DETAILED DESCRIPTION OF THE INVENTION

[00024] An isolated polynucleotide and an isolated polypeptide, as used herein, can be isolated from its natural environment or can be synthesized. Complete purification is not required in either case. Amino acid and nucleotide sequences flanking an isolated polypeptide or polynucleotide that occurs in nature, respectively, can but need not be absent from the isolated form.

[00025] Further, an isolated polynucleotide has a structure that is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term includes, without limitation, (a) a nucleic acid molecule having a sequence of a naturally occurring genomic or extrachromosomal nucleic acid molecule but which is not flanked by the coding sequences that flank the sequence in its natural position; (b) a nucleic acid molecule incorporated into a vector or into a prokaryote or

eukaryote genome such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids present in mixtures of clones, e.g., as these occur in a DNA library such as a cDNA or genomic DNA library. An isolated nucleic acid molecule can be modified or unmodified DNA or RNA, whether fully or partially single-stranded or double-stranded or even triple-stranded. A nucleic acid molecule can be chemically or enzymatically modified and can include so-called non-standard bases such as inosine.

[00026] Reference herein to use of AT is understood to encompass use of an ATR-binding component thereof, especially PA.

Anthrax Toxin Receptor

[00027] The applicants have identified and determined the nucleic acid sequence (SEQ ID NO:1) of a cDNA clone that of a 368 amino acid long polypeptide (SEQ ID NO:2, *ATR*), and show herein that the polypeptide is a surface-bound anthrax toxin receptor (ATR) on human cells. Based on known structural analysis methods, the polypeptide is predicted to encode a 27 amino-acid-long signal peptide (amino acids 1-27 of SEQ ID NO:2), a 293 amino-acid-long extracellular domain (amino acids 28-320 of SEQ ID NO:2), a 23 amino-acid-long putative transmembrane region (amino acids 320-343 of SEQ ID NO:2), and a 25 amino acid long cytoplasmic domain (amino acids 344-368 of SEQ ID NO:2).

[00028] It is disclosed herein that Protective Antigen (PA) of anthrax toxin (AT) binds to the anthrax toxin receptor at a von Willebrand factor A (VWA) domain located in the portion from amino acid 44 to 216 in the extracellular domain of SEQ ID NO:2. VWA domains are present in the extracellular portions of a variety of cell surface proteins, including matrilins and integrins (designated as I domains). A VWA domain consensus sequence, VWA-CON, developed by comparing 210 related sequences, is presented as SEQ ID NO:3. These domains are important for protein/protein interactions and constitute ligand binding sites for integrins (Dickeson, 1998). The I domain of integrin $\alpha 2$ ($\alpha 2$) is presented as SEQ ID NO:4. Ligand binding through I domains requires an intact metal ion-dependent adhesion site (MIDAS) motif (Lee,

1995) which appears to be conserved in the ATR extracellular domain, as is detailed below.

[00029] Comparison of SEQ ID NO:1 and SEQ ID NO:2 to existing databases revealed other versions of those sequences. Human cDNA TEM8 (SEQ ID NO:5; Genbank accession number NM 032208) encodes a 564 amino-acid-long form (SEQ ID NO:6) of the human ATR. SEQ ID NO:6 has not previously been identified as an anthrax toxin receptor, and indeed no function has yet been ascribed to the protein. Like SEQ ID NO:1, SEQ ID NO:5 was a PCR amplification product from HeLa cells and human placenta cDNA libraries. Whereas the cytoplasmic tail of SEQ ID NO:2 is only 25 amino acids long, that of SEQ ID NO:6 is predicted to be 221 amino acids long (amino acids 344-564), presumably as a result of differential splicing of a primary mRNA transcript. The proteins are otherwise identical. Upstream of the coding sequences, SEQ ID NO:1 and SEQ ID NO:5 are also identical.

[00030] Also presented are IMAGE CLONE 4563020 (SEQ ID NO:7; Genbank Accession Number BC012074) and the predicted polypeptide encoded by the clone (SEQ ID NO:8). SEQ ID NO:8 is identical to amino acids 1-317 of ATR, but differs thereafter at the C-terminus. Similarly, human cDNA FLJ10601, clone NT2RP2005000 (SEQ ID NO:9; Genbank Accession Number AK001463) and the predicted polypeptide encoded by the clone (SEQ ID NO:10) are presented. This polypeptide is identical to a portion of SEQ ID NO:2 from amino acid 80 to amino acid 218. As with TEM8 and the protein it encodes, no function is known for any of these polynucleotide and polypeptide sequences, nor has there been any prior indication that the polypeptides are complete or partial anthrax toxin receptors.

[00031] It is of interest to note that the product of the mouse homolog of ATR/TEM8 (Genbank accession number AK013005) is highly related to the human clones, sharing greater than 98% amino acid sequence identity within the reported extracellular domain. This suggests that the anthrax toxin receptor is conserved among species. Furthermore, consistent with the observation that the anthrax toxin receptor is found in a variety of cell lines, *ATR* is expressed in a number of different tissues including CNS, heart, lung, and lymphocytes.

[00032] In addition to the full-length and partial ATR polypeptide sequences presented in SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10, other polypeptide fragments shorter than those sequences that retain PA-binding activity, and variants thereof are also within the scope of the invention. The entire receptor is not required for utility; rather, fragments that

bind to PA are useful in the invention.

[00033] A skilled artisan can readily assess whether a fragment binds to PA. A polypeptide is considered to bind to PA if the equilibrium dissociation constant of the binary complex is 10 micromolar or less. PA-binding to the ATR (or a fragment of the ATR) can be measured using a protein-protein binding method such as coimmunoprecipitation, affinity column analysis, ELISA analysis, flow cytometry or fluorescence resonance energy transfer (FRET), and surface plasmon resonance (SPR). SPR is particularly suited as it is highly sensitive and accurate, operable in real time, and consumes only minute amounts of protein. SPR uses changes in refractive index to quantify macromolecular binding and dissociation to a ligand covalently tethered to a thin gold chip in a micro flow cell. Besides the equilibrium dissociation constant (Kd), on- and off-rate constants (ka and kd) can also be obtained. A BIAcore 2000 instrument (Pharmacia Biotech) can be used for these measurements. Typically, a protein is covalently tethered to a carboxymethyl dextran matrix bonded to the gold chip. Binding of a proteinaceous ligand to the immobilized protein results in a quantifiable change in refractive index of the dextran/protein layer. SPR can also be used to determine whether the interaction between PA and its receptor is sensitive to low pH, which is relevant to toxin endocytosis. This technique has been used to study protein-protein interactions in many systems, including the interactions of PA63 with EF and LF (Elliott, 1998).

[00034] The invention also relates to polypeptides that are at least 80%, preferably at least 90%, more preferably at least 95%, still more preferably at least 97%, or most preferably at least 99% identical to any aforementioned PA-binding polypeptide fragment, where PA-binding is maintained. As used herein, "percent identity" between amino acid or nucleic acid sequences is synonymous with "percent homology," which can be determined using the algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA 87:2264-2268, 1990), modified by Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90:5873-5877, 1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (J. Mol. Biol. 215:403-410, 1990). BLAST nucleotide searches are performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleic acid molecule of the invention. BLAST protein searches are performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a reference polypeptide (e.g., SEQ ID NO:2). To obtain

gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul et al. (Nucleic Acids Res. 25:3389-3402, 1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used. See <http://www.ncbi.nlm.nih.gov>. A variant can also include, e.g., an internal deletion or insertion, a conservative or non-conservative substitution, or a combination of these variations from the sequence presented.

[00035] Soluble fragments are of great interest as these can competitively inhibit anthrax toxin binding to the ATR and thereby can protect cells from AT intoxication *in vivo* and *in vitro*. A fragment is soluble if it is not membrane-bound and is soluble in an aqueous fluid. The extracellular ATR domain is a soluble fragment of the ATR, as are fragments of that domain. Even though the VWA domain is formally identified as extending from amino acid 44 to 216 in the extracellular domain, more or fewer natively adjacent amino acids can be included in the fragment without compromising solubility or PA-binding. For example, a PA-binding fragment having the sequence of SEQ ID NO:2 beginning at any amino acid in the range from 27 to 43 and ending at any amino acid in the range from 221 to 321. A preferred soluble, PA-binding fragment extends from amino acid 42 to 222. Another preferred soluble PA-binding fragment includes a fragment of the ATR from amino acid 27 through amino acid 321. Likewise, any polypeptide fragment of these preferred fragments that retains PA-binding activity is within the scope of the invention. ATR in soluble form is effective in a monomeric form, as well as in multimeric forms such as dimeric, tetrameric, pentameric and higher oligomeric forms.

[00036] PA-binding polypeptides can include, therefore, SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, a PA-binding fragment of SEQ ID NO:2, a PA-binding fragment of SEQ ID NO:6, a PA-binding fragment of SEQ ID NO:8, a PA-binding fragment of SEQ ID NO:10, a PA-binding polypeptide at least 80% identical to any of the foregoing fragments. The PA-binding polypeptides can also be provided as fusion proteins comprising any of the foregoing that can comprise still other non-natively adjacent amino acids for detecting, visualizing, isolating, or stabilizing the polypeptide. For example, PA binds to a soluble fusion protein of a hexahistidine tag, a T7 tag, and amino acids 41-227 of ATR.

[00037] Likewise, isolated polynucleotides having an uninterrupted nucleic acid sequence that encodes the aforementioned polypeptides and polypeptide fragments are also useful in the

invention. The sequences that encode soluble, PA-binding polypeptide fragments of ATR are immediately apparent to the skilled artisan from the description of the relevant portions of the polypeptides, *supra*. An isolated nucleic acid containing the complement of any such polynucleotide is also within the scope of the present invention, as are polynucleotide and oligonucleotide fragments for use as molecular probes. The polynucleotides of the invention cannot encode SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10.

[00038] The present invention also relates to an isolated polynucleotide and its complement, without regard to source, where the polynucleotide hybridizes under stringent or moderately stringent hybridization conditions to SEQ ID NO:1, SEQ ID NO:5, SEQ ID 7, or SEQ ID NO:9 or to a fragment of any of the foregoing that encodes a soluble polypeptide that can bind to PA. As used herein, stringent conditions involve hybridizing at 68°C in 5x SSC/5x Denhardt's solution/1.0% SDS, and washing in 0.2x SSC/0.1% SDS +/- 100 µg/ml denatured salmon sperm DNA, at room temperature. Moderately stringent conditions include washing in the same buffer at 42°C. Additional guidance regarding such conditions is readily available in the art, for example, by Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; and Ausubel et al. (eds.), 1995, Current Protocols in Molecular Biology, (John Wiley & Sons, N.Y.) at Unit 2.10.

[00039] In a related aspect, any polynucleotide of the invention can be provided in a vector in a manner known to those skilled in the art. The vector can be a cloning vector or an expression vector. In an expression vector, the polypeptide-encoding polynucleotide is under the transcriptional control of one or more non-native expression control sequences, such as a promoter not natively adjacent to the polynucleotide, such that the encoded polypeptide can be produced when the vector is delivered into a compatible host cell that supports expression of an polypeptide encoded on a vector, for example by electroporation or transfection, or transcribed and translated in a cell-free transcription and translation system. Such cell-based and cell-free systems are well known to the skilled artisan. Cells comprising an insert-containing vector of the invention are themselves within the scope of the present invention, without regard to whether the vector is extrachromosomal or integrated in the genome.

[00040] A skilled artisan in possession of the polypeptides and polynucleotides of the invention can also identify agents that can reduce or prevent the effect of AT on a host having on

the cell surface at least a portion of the ATR. The effect altered can relate, for example, to (1) susceptibility of the host cell to AT damage, (2) integration of ATR into the cell membrane, (3) binding between ATR and PA, (4) PA heptamerization, (5) uptake of PA and ATR complex into cells, and (6) the translocation of toxin into host cell cytoplasm. The method includes separately exposing a plurality of putative agents in the presence of AT to a plurality of cells, comparing the effect of AT on the cells in the presence and absence of the agent, and identifying at least one agent that alters an effect of AT on the cells.

[00041] The skilled artisan can readily evaluate the typical effects of AT and can observe variations in those effects in the presence of a putative altering agent. For example, susceptibility to AT damage can be evaluated by exposing host cells to AT. Integration of newly formed ATR into the host cell membrane can be evaluated by labeling newly synthesized proteins in the host cell and immunoprecipitating ATR from the cellular membrane fraction of the host cell. Binding of wild-type ATR to PA can be evaluated with fluorescent labeled anti-PA antibody. PA heptamerization can be evaluated by several techniques including native polyacrylamide gel electrophoresis, gel filtration, and western blotting. Uptake of PA-ATR complex can be evaluated by binding PA to ATR at 4°C, increasing the temperature to 37°C to allow endocytosis, shifting the temperature back to 4°C, and incubating cells with fluorescent labeled anti-PA antibodies. Toxin translocation into the host cell cytoplasm can be evaluated as described in Wesche et al, 1998, which is incorporated herein by reference as if set forth in its entirety.

[00042] The agents screened can be, for example, dominant negative mutant ATRs (encoded by a mutant polynucleotide sequence, which can be provided in an expression vector), a high molecular weight molecule such as a polypeptide (including, e.g., a mutant AT, a soluble ATR, a mono- or polyclonal antibody to an ATR, to PA, or to an ATR/PA complex), a polysaccharide, a lipid, a nucleic acid, a low molecular weight organic or inorganic molecule, or the like. Antibodies can be produced by administering to a non-human animal an immunogenic, PA-binding fragment of a polypeptide which can be, e.g., SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, a polypeptide at least 80% identical to any of the foregoing and a fusion protein comprising any of the foregoing, and then obtaining the desired antibodies using known methods.

[00043] Chemical libraries for screening putative agents, including peptide libraries, are

readily available to the skilled artisan. Examples include those from ASINEX (i.e. the Combined Wisdom Library of 24,000 manually synthesized organic molecules) and from CHEMBRIDGE CORPORATION (i.e. the DIVERSet™ library of 50,000 manually synthesized chemical compounds; the SCREEN-Set™ library of 24,000 manually synthesized chemical compounds; the CNS-Set™ library of 11,000 compounds; the Cherry-Pick™ library of up to 300,000 compounds) and linear library, multimeric library and cyclic library (Tecnogen (Italy)). Once an agent with desired activity is identified, a library of derivatives of that agent can be screened for better agents. Phage display is also a suitable approach for finding novel inhibitors of the interaction between PA and ATR.

[00044] Another aspect of the present invention relates to ATR ligands other than PA and methods for identifying ATR ligands. As ATR is expressed in many cell types, it likely has other natural ligands. To identify these other ligands, a polypeptide that contains an ATR VWA domain, preferably an entire extracellular domain can be provided in soluble or tethered form, e.g., in a chromatographic column. Preferably, the ectodomain of ATR can be provided as a fusion protein that also contains rabbit IgG constant region, a GST domain or a hexahistidine tag. This fusion protein can be immobilized on a chromatographic column using known methods. A cell extract can be passed over the column. A ligand is identified when binding is observed between the ectodomain and a compound present in the cell extract. The identified ligand can be used in methods for identifying agents that alter an effect of AT, to identify an agent that selectively inhibits PA-ATR binding. It is also desirable to use the other ligands and the ATR in comparative high throughput screening methods for identifying small molecules that do not interfere with natural ligand binding to ATR, but which do prevent or reduce binding of ATR to anthrax toxin.

[00045] The present invention also relates to reducing cellular damage caused by AT, which can be achieved by administering an agent for reducing the ATR level, inhibiting the binding between ATR and AT, or by reducing downstream ATR activity after AT binding. For example, an antisense oligonucleotide can reduce or prevent expression of *atr* using delivery methods known to the skilled artisan, thus reducing the cellular ATR level. An ATR-anthrax binding inhibition agent can inhibit the binding between ATR and AT. Dominant negative ATRs can block downstream ATR activities required for AT toxicity. The agents used for reducing AT

damage to cells can be administered to a human or non-human animal, preferably in a standard pharmaceutical carrier, in an amount effective to reduce or eliminate anthrax toxicity.

[00046] A 20-25mer antisense oligonucleotide can be directed against 5' end of the *atr* message with phosphorothioate derivatives on the last three base pairs on the 3' end and the 5' end to enhance the half life and stability of the oligonucleotides. A carrier for an antisense oligonucleotide can be used. An example of a suitable carrier is cationic liposomes. For example, an oligonucleotide can be mixed with cationic liposomes prepared by mixing 1-alpha dioleylphatidylcelthanolamine with dimethldioctadecylammonium bromide in a ratio of 5:2 in 1 ml of chloroform. The solvent will be evaporated and the lipids resuspended by sonication in 10 ml of saline. Another way to use an antisense oligonucleotide is to engineer it into a vector so that the vector can produce an antisense cRNA that blocks the translation of the mRNAs encoding for ATR. Similarly, RNAi techniques, which are now being applied to mammalian systems, are also suited for inhibiting ATR expression (see Zamore, *Nat. Struct. Biol.* 8:746:750 (2001), incorporated herein by reference as if set forth in its entirety).

[00047] The present invention also relates to a method for detecting *atr* mRNA or ATR protein in a sample. Such detection can be readily accomplished by using oligonucleotide or polynucleotide probes for *atr* mRNA, or antibodies for ATR protein. In a related aspect, the antibodies made and identified as being able to bind to ATR can also be used to separate ATR from a sample.

[00048] The present invention also relates to a cell line that does not contain ATR from a parent cell line that contains ATR, and methods for making same. The present invention provides that it is possible for cells lacking ATR to survive. In the example described below, a cell line that does not contain ATR was created using mutagenesis and screening. Now that the *atr* cDNA sequence is identified in the present invention, many other methods for generating a cell line that does not express *atr* become feasible, such as homologous recombination. In addition to these methods, the cell lines generated, including the one described in the example below, are themselves within the scope of the present invention.

[00049] The invention also provides molecules and methods for specifically targeting and killing cells of interest by delivering, e.g., AT or LF to the cell. Soluble ATR molecules can be coupled to a ligand or to a single chain antibody selected for targeting to the cell of interest (e.g.,

a ligand that binds a receptor presented on a tumor cell surface). The coupling is most readily accomplished by producing a fusion protein that encodes both the ATR binding portion and the ligand or single chain antibody molecule. The ligand or single chain antibody domains simply serve to attach the toxin to cells with the cognate surface markers. The toxin or factor is preloaded onto the ATR portion before exposing the coupled molecules to the targeted cells. This is similar in principle to the previously described for retroviral targeting using soluble retroviral receptor-ligand bridge proteins and retroviral receptor-single chain antibody bridge proteins. See Snitkovsky and Young, Proc. Natl. Acad. Sci. USA 95:7063-7068 (1998); Boerger et al. Proc. Natl. Acad. Sci. USA 96:9687-9872 (1999) and Snitkovsky et al., J. Virol. 74:9540-9545 (2000), and Snitkovsky et al., J. Virol. 75:1571-1575 (2001), each incorporated herein by reference as if set forth in its entirety.

[00050] The invention will be more fully understood upon consideration of the following non-limiting examples.

EXAMPLES

Methods*Mutagenesis and characterization of CHO-K1 cells*

[00051] A mutant cell line lacking the receptor was generated, so that this defect could be genetically complemented. About 5×10^7 cells of the hypodiploid CHO-K1 cell line were treated at 37°C for 7 hr with medium containing 10 $\mu\text{g}/\text{ml}$ ICR-191 (Sigma), a DNA alkylating agent that induces small deletions and frameshift mutations in genes, then washed twice. This treatment led to approximately 90% cell death.

[00052] The surviving mutagenized cells were then challenged with 8 $\mu\text{g}/\text{ml}$ PA and 10 ng/ml LF_N-DTA, a fusion protein composed of the N-terminal 255 amino acids of LF linked to the catalytic A chain of diphtheria toxin. This recombinant toxin can kill CHO-K1 cells (in contrast to LF and PA) and it exploits the same LF/PA/receptor interactions that are required for the binding and entry of the native LF and EF proteins. After 4 days, surviving cells were replated and incubated for 3 days with medium containing PA and LF_N-DTA. Ten single-cell colonies (designated as CHO-R1.1 to CHO-R1.10) that survived toxin treatment were isolated 14 days later. In control experiments performed with non-mutagenized CHO-K1 cells, no toxin-resistant cell clones were detected.

[00053] One of the mutagenized clones (CHO-R1.1) was chosen for further analysis. CHO-R1.1 cells were found to be fully susceptible to killing by diphtheria toxin (DT) by measuring ³H-leucine incorporation into cellular proteins after exposure to the toxin, thus ruling out the possibility that resistance to PA/LF_N-DTA was due to a defect in the pathway of DT action. To test directly whether CHO-R1.1 cells lacked the receptor, flow cytometric analysis was performed after the cells were incubated at 4°C for 2 hr in medium containing 40 to 80 nM PA-K563C coupled at mutated residue 563 to Oregon Green maleimide (Molecular Probes) ("OGPA"). The treated cells were washed twice with medium and analysed using a Becton Dickinson FACSCalibur flow cytometer. CHO-R1.1 cells were significantly impaired in their ability to bind to OGPA as compared to the parental cell line, suggesting that these mutagenized cells had lost expression of the putative PA receptor gene. Similar analysis of the other nine mutant CHO-R1 clones demonstrated that they were also defective in binding to OGPA.

cDNA complementation

[00054] In an attempt to complement the PA binding defect of CHO-R1.1 cells, the cells were transduced with a retrovirus-based cDNA library (Clontech) prepared from human HeLa cells that express the PA receptor. This cDNA library is contained in a murine leukemia virus (MLV) vector that is packaged into pseudotyped virus particles (MLV[VSV-G]) containing the broad host-range G protein of vesicular stomatitis virus (VSV-G). Retrovirus-based cDNA libraries are useful for genetic complementation approaches since they can deliver a limited number of stably expressed cDNA molecules per cell. These molecules can be rapidly re-isolated by PCR amplification using MLV vector-specific oligonucleotide primers.

[00055] Approximately 5×10^5 CHO-R1.1 cells were transduced with about 10^7 infectious units (complexity of library = 2×10^6 independent clones) of the pLIB-based cDNA library (Clontech; cat.# HL8002BB) produced in the 293GPG packaging cell line. Three days later, cells were incubated with medium containing 80 nM OGPA and the top 0.1% of fluorescent cells were then isolated by sorting using a Becton Dickinson FACSVantageSE instrument. Cells were sorted based on their binding of OGPA in combination with an anti-PA polyclonal serum and an allophycocyanin (APC) conjugated secondary antibody. To isolate those that contained the putative PA receptor cDNA clone, these cells were expanded and subjected to four additional rounds of sorting using OGPA as above, as well as a 1:500 dilution of a rabbit anti-PA polyclonal serum along with a 1:500 dilution of an APC-conjugated secondary antibody (Molecular probes). OGPA-single positive (round 2) or OGPA/APC-double positive (rounds 3-5) cells were recovered (the top 20%, 1%, 5%, and 50% of fluorescent cells for rounds 2, 3, 4, and 5 respectively) and expanded after each round of sorting.

[00056] This led to the isolation of a cell population in which greater than 90% of the cells bound OGPA. This complemented cell population contained at least seven unique cDNA inserts that were obtained by the PCR amplification method described above. Each cDNA was gel purified, subcloned back into the parent pLIB vector and packaged into MLV(VSV-G) virions so that it could be tested for its ability to complement the PA-binding defect of CHO-R1.1 cells. One cDNA clone of approximately 1.5 kb (designated as *ATR*) restored PA binding to CHO-R1.1 cells. This clone also dramatically enhanced the binding of PA to parental CHO-K1 cells.

[00057] Furthermore, the *ATR* cDNA clone fully restored LF_N-DTA/PA toxin sensitivity to CHO-R1.1 cells. In this test, CHO-R1.1 cells and CHO-K1 cells were either not transduced or

transduced with the MLV vector encoding ATR; these cells were treated with 10^{-9} M LF_N-DTA and various concentrations of PA; medium containing 1 μ Ci/mL 3 H-leucine was then added to cells for 1 hr, and the amount of 3 H-leucine incorporated into cellular proteins was determined by trichloroacetic acid precipitation and liquid scintillation counting.

CDNA characterization

[00058] cDNA inserts were recovered from these cells by PCR amplification of genomic DNA samples using oligonucleotide primers specific for the MLV vector according to the manufacturers instructions (Clontech). Each cDNA was subcloned between the *Not*I and *Sal*I restriction enzyme sites of pLIB and the resulting plasmids were co-transfected into 293 cells with MLV gag/pol and VSV-G expression plasmids pMD.old.gagpol and pMD.G. Resulting pseudotyped virus particles were used to infect CHO-R1.1 and CHO-K1 cells followed by OGPA staining and FACS analysis as above.

[00059] Sequencing of the *ATR* cDNA clone revealed a single long open reading frame, encoding a 368 amino acid protein. Fig. 1 shows sequence alignment of ATR (SEQ ID NO:2) with the von Willebrand factor A domain consensus sequence (SEQ ID NO:3; VWA-CON), the I domain of integrin $\alpha 2$ (SEQ ID NO:4; $\alpha 2$), and TEM8 (SEQ ID NO:6). The secondary structural elements are based on the crystal structure of the $\alpha 2$ I domain. Conserved amino acids are boxed and identical amino acids are indicated by shaded boxes. The putative signal sequence is underlined. The five residues that form the MIDAS motif are indicated with asterisks. The putative transmembrane domains of ATR and TEM8 are indicated with a shaded box. Potential N-linked glycosylation sites in ATR and TEM8 are indicated by hatched boxes. The alignment was made using the programs ClustalW and ESPript 1.9.

[00060] The ATR protein is predicted to have a 27 amino acid long signal peptide, a 293 amino acid long extracellular domain with three putative N-linked glycosylation sites, a 23 amino acid long putative transmembrane region, and a short cytoplasmic tail. A BLAST search revealed that the first 364 amino acids of ATR are identical to a protein encoded by the human *TEM8* cDNA clone (Genbank accession number NM 032208). The C-terminal ends of ATR and the TEM8 protein then diverge, presumably as a consequence of alternative splicing, such that ATR has a cytoplasmic tail of only 25 amino acids whereas TEM8 is predicted to have a 221 amino acid long cytoplasmic tail. The most notable feature of ATR is the presence of an

extracellular von Willebrand Factor type A (VWA) domain, located between residues 44 and 216.

[00061] The cytoplasmic tail of ATR contains an acidic cluster (AC motif) (EESEE) that is similar to a motif found in the cytoplasmic tail of furin which specifies basolateral sorting of this protease in polarized epithelial cells. This may be significant because the PA receptor localizes to the basolateral surface of polarized epithelial cells and it is expected that the receptor and the protease needed to bind and activate PA would be co-localized to allow for efficient entry of anthrax toxins.

Cloning and Expression of T7-ATR₄₁₋₂₂₇

[00062] A fusion protein having a hexahistidine tag, a T7 tag, and amino acids 41 to 227 of ATR (the I domain) was constructed, expressed and purified from *E. coli* cells as follows. A DNA fragment encoding amino acids 41-227 of ATR was cloned into the *Bam*H1 and *Eco*R1 sites of pET28A (Novagen) to generate pET28A-ATR₄₁₋₂₂₇. BL21 (DE3) cells (Stratagene) containing pET28A-ATR₄₁₋₂₂₇ were grown at 37°C to an OD₆₀₀ of 0.6, induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 4 hr and harvested by centrifugation. The cells from 1.5 L of culture were resuspended in 25 mL of 50 mM Tris-HCl pH 8.0, 2 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride and were passed through a French press. One milligram of DNase I (Roche) was added to the cell lysate, which was then sonicated for 1 min and centrifuged at 21,000 g for 20 min. The pellet was resuspended in 25 mL of 50 mM Tris-HCl pH 8.0, 2 mM DTT and centrifuged at 21,000 g for 20 min. This wash step was repeated once. T7-ATR₄₁₋₂₂₇ was solubilized and folded essentially as described previously.

[00063] When mixed with wild-type PA (on ice for 30 min), this construct was precipitated with polyclonal anti-PA serum (analyzed by SDS-PAGE and Western blot using anti-T7 antibody conjugated to horseradish peroxidase). The interaction between PA and T7-ATR₄₁₋₂₂₇ was impaired by the presence of EDTA (2 mM), demonstrating that the involvement of divalent cations in the interaction, and suggesting that the ATR MIDAS motif is involved in binding PA.

Interaction between PA and ATR

[00064] PA-N682S, a mutant form of PA isolated as described below and having an impaired ability to bind and intoxicate cells, did not bind to T7-ATR₄₁₋₂₂₇. The DNA encoding

Domain 4 of PA was mutagenized using error-prone PCR. Clones were expressed in *E. coli*, and lysates derived from these clones were added to CHO-K1 cells in combination with LF_N-DTA. Clones corresponding to lysates that did not kill CHO-K1 cells were sequenced and the N682S mutant clone was further characterized as having Ser in place of Asn at position 682.

[00065] PA-N682S was shown to have an impaired ability to bind cells as follows: CHO-K1 cells were incubated with 2x10⁻⁸ M trypsin-nicked PA (wild-type or N682S) for 1 hr, washed with PBS, resuspended in SDS sample buffer and run on a 4-20 % polyacrylamide SDS gel, and PA was visualized by Western blotting. In the experiment in which PA-N682S was shown to have an impaired ability to intoxicate cells, CHO-K1 cells were incubated with LF_N-DTA (10⁻⁹ M) and various concentrations of wild-type PA or PA-N682S mutant, and cell viability was determined.

[00066] To confirm that PA binds directly to ATR, co-immunoprecipitations (using a polyclonal serum specific for PA and protein A agarose) were performed with an extracellular fragment of ATR and either the wild-type or a receptor binding-deficient mutant form of PA. A mixture of 5 µg PA (WT or N682S) and 2 µg T7-ATR₄₁₋₂₂₇ (in 20 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1 mg bovine serum albumin per mL) was incubated on ice for 30 min in the presence or absence of 2 mM EDTA. Anti-PA polyclonal serum (10 µL) was added to this solution and incubated on ice for an additional 1 hr. Protein A agarose (Santa Cruz Biotechnology) was added and the solution was rotated at 4°C for 1 hr, then washed four times with 20 mM Tris-HCl pH 8.0, 150 mM NaCl. Approximately one third of the mixture was subjected to SDS-PAGE, transferred to nitrocellulose and probed with anti-T7 antibody conjugated to horseradish peroxidase (Novagen).

[00067] In addition, a fusion protein containing GST and the PA receptor-binding domain (D4) (GST-D4) bound T7-ATR₄₁₋₂₂₇, while GST did not. DNA encoding amino acids 595 to 735 of PA (domain 4) was cloned into pGEX-4T-1 (Pharmacia Biotechnology). This vector encoded the GST-D4 fusion protein. GST-D4 was coupled to glutathione sepharose at a concentration of 4 mg GST-D4 per mL according to manufacturer's instructions (Pharmacia Biotechnology). GST or GST-D4 coupled to glutathione sepharose was mixed with 2 µg of T7-ATR₄₁₋₂₂₇ and 250 µg of *E. coli* extract in a volume of 250 µL for 1 hr at 4°C. The beads were washed 4 times with 20 mM Tris-HCl pH 8.0, 150 mM NaCl. One half of the suspension was subjected to SDS-

PAGE, transferred to nitrocellulose, and probed with anti-T7 antibody coupled to horseradish peroxidase.

[00068] Taken together, the experiments described above demonstrate a direct and specific interaction between the VWA/I domain of ATR and the receptor-binding domain of PA. Given this direct interaction, we reasoned that ATR₄₁₋₂₂₇ might protect CHO-K1 cells from killing by PA and LF_N-DTA. This idea was tested by incubating (37°C for 4 hr) CHO-K1 cells with an increasing amount of T7-ATR₄₁₋₂₂₇ in the presence of a constant amount of PA (10⁻¹⁰ M)/LF_N-DTA (2.5 × 10⁻¹¹ M), and then measuring the subsequent effect on protein synthesis. T7-ATR₄₁₋₂₂₇ was an effective inhibitor of toxin action, inhibiting toxin activity by 50% and 100% at concentrations of 80 nM and 500 nM respectively. T7-ATR₄₁₋₂₂₇ did not, however, inhibit diphtheria toxin.

[00069] The present invention is not intended to be limited to the foregoing, but encompasses all such modifications and variations as come within the scope of the appended claims.

CLAIMS

WE CLAIM:

1. A isolated polypeptide selected from the group consisting of SEQ ID NO:2, a PA-binding fragment of SEQ ID NO:2, a PA-binding fragment of SEQ ID NO:6, a PA-binding fragment of SEQ ID NO:8, a PA-binding fragment of SEQ ID NO:10, a PA-binding polypeptide at least 80% identical to any of the foregoing fragments, and a fusion protein comprising any of the foregoing.
2. The isolated polypeptide of claim 1 wherein the polypeptide is a soluble polypeptide.
3. The isolated polypeptide of claim 1, wherein the PA-binding fragment of SEQ ID NO:2 begins at any amino acid in the range from 27 to 43 and ends at any amino acid in the range from 221 to 321.
4. The isolated polypeptide of claim 1 having an amino acid sequence set forth in SEQ ID NO:2.
5. An isolated polynucleotide or complement thereof, the polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NO:2, a PA-binding fragment of SEQ ID NO:2, a PA-binding fragment of SEQ ID NO:6, a PA-binding fragment of SEQ ID NO:8, a PA-binding fragment of SEQ ID NO:10, a PA-binding polypeptide at least 80% identical to any of the foregoing fragments, and a fusion protein comprising any of the foregoing, the polynucleotide being unable to encode SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10.

6. The isolated polynucleotide of claim 5, wherein the PA-binding fragment of SEQ ID NO:2 begins at any amino acid in the range from 27 to 43 and ends at any amino acid in the range from 221 to 321.

7. The isolated polynucleotide of claim 5 comprising SEQ ID NO:1 from position 104 to 1207 or the complement thereof.

8. The isolated polynucleotide of claim 5 comprising SEQ ID NO:1 or the complement thereof.

9. The isolated polynucleotide of claim 5, wherein the polynucleotide encodes a soluble polypeptide.

10. An isolated polynucleotide or complement thereof, the polynucleotide hybridizing under stringent or moderately stringent hybridization conditions to all or a portion of SEQ ID NO:1 and encoding a soluble, PA-binding polypeptide.

11. A vector comprising a polynucleotide selected from the group consisting of a polynucleotide of claim 5 and a polynucleotide of claim 10.

12. The vector of claim 11, further comprising a non-native expression control sequence operably linked to the polynucleotide.

13. A host cell comprising a vector of claim 11.

14. A method for making an antibody, the method comprising the step of: administering to a non-human animal an immunogenic, PA-binding fragment of a polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, a polypeptide

at least 80% identical to any of the foregoing and a fusion protein comprising any of the foregoing.

15. A method for identifying an agent that inhibits binding between protective antigen (PA) and anthrax toxin receptor, the method comprising the steps of:

combining protective antigen (PA) and a polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, a PA-binding fragment of SEQ ID NO:2, a PA-binding fragment of SEQ ID NO:6, a PA-binding fragment of SEQ ID NO:8, a PA-binding fragment of SEQ ID NO:10, a PA-binding polypeptide at least 80% identical to any of the foregoing, and a fusion protein comprising any of the foregoing, separately with and without a putative binding-inhibiting agent;

comparing binding between PA and the polypeptide with and without the putative agent; and

identifying a decrease in binding with the putative agent, the decrease being an indication that the test agent inhibits the binding of PA to the anthrax toxin receptor.

16. A method for treating anthrax in a human or non-human animal, the method comprising the step of:

administering to the animal an agent that inhibits binding between protective antigen (PA) and anthrax toxin receptor at a level effective to reduce the severity of anthrax.

17. A method as claimed in claim 16, wherein the agent that inhibits binding between PA and the anthrax toxin receptor is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, a PA-binding fragment of SEQ ID NO:2, a PA-binding fragment of SEQ ID NO:6, a PA-binding fragment of SEQ ID NO:8, a PA-binding fragment of SEQ ID NO:10, a PA-binding polypeptide at least 80% identical to any of the foregoing, a fusion protein comprising any of the foregoing, a monoclonal antibody, a polyclonal antibody, a polysaccharide, a lipid, and a nucleic acid.

18. A cultured cell having a cell membrane having an exterior surface, the exterior surface displaying no receptor for anthrax toxin protective antigen.

19. A method for producing an anthrax toxin receptor, the method including the step of: transcribing a polynucleotide that encodes an anthrax toxin receptor operably linked to an upstream expression control sequence, the receptor being selected from the group consisting of SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, a PA-binding fragment of SEQ ID NO:2, a PA-binding fragment of SEQ ID NO:6, a PA-binding fragment of SEQ ID NO:8, a PA-binding fragment of SEQ ID NO:10, a PA-binding polypeptide at least 80% identical to any of the foregoing, and a fusion protein comprising any of the foregoing, to produce an mRNA; and translating the mRNA to produce the anthrax toxin receptor.

20. A method as claimed in Claim 19, wherein the polynucleotide is operably linked to the expression control sequence in an expression vector, and wherein the expression vector is delivered into a host cell, the expression control sequence being operable in the host cell.

21. A method as claimed in Claim 19, wherein at least one of the transcribing and translating steps are performed *in vitro*.

1/1

α2-1 β_1 α_1
000000 000000
α2-1 1
VWA-CON 1
TEM8 1 MATAKRALGIGFQWLSSLATLVLICAGQGRRREDDGGPACYGGFELVFLDNGS...
ATR 1 MATAKRALGIGFQWLSSLATLVLICAGQGRRREDDGGPACYGGFELVFLDNGS...

α2-1 β_2 β_3 α_2 α_3 η_1 β_4
α2-1 41 MVOGLIQYANSPRKVVFVNTYATKEKMTVAFSQTS. DYCSDIHTFGA101PRKAYGAAZGGERGQTYEVVVVDEGSH
VWA-CON 40 DPGVGLVTPSSDARVLFPEHDSQSKEALLEAALANLSSLSG9. DHLGAALVYVLENLYSEHAGSRRECPKLLILIDLSS
TEM8 78 IEMSPFLVFSSTRGTTLMKTED. . REQIROGELBLQVLPFGDNTYHEGPERSEQTYTENRQGY. TASVTLALGKSH
ATR 78 IEMSPFLVFSSTRGTTLMKTED. . REQIROGELBLQVLPFGDNTYHEGPERSEQTYTENRQGY. TASVTLALGKSH

α2-1 η_2 α_4 β_5 α_5 α_6 α_7 β_6 η_3 α_8
α2-1 120 DGS...SMILKAVIDQCNEDNILRQDIAVGLYTLRNALDTRKLNLIKEIKAJDSDPTERYPTEVSDREAMJERAGTEGRQIIFSIE
VWA-CON 119 DGGEDILKAAKELTS.
TEM8 155 ED..LFFYISREKANRSR... DICAQIYCVGUK. DPNET... QLARTEDISKDHEVPPVNDGFOALQALQIISIISKSCIEIL
ATR 155 ED..LFFYISREKANRSR... DICAQIYCVGUK. DPNET... QLARTEDISKDHEVPPVNDGFOALQALQIISIISKSCIEIL

α2-1 198 G
TEM8 225 AARPSTICAGESPQVVVRGNGPRHARNVDRVLCSFKINDSVTLNEKPPSVEDTYLLCPAPILKEVGMKAALQVSMNDGLS
ATR 225 AARPSTICAGESPQVVVRGNGPRHARNVDRVLCSFKINDSVTLNEKPPSVEDTYLLCPAPILKEVGMKAALQVSMNDGLS
TEM8 300 FISSSVIITTTCHSDGSILALALLLFLILLALALLWWFWPLCCTVIIKEVPPPPARESHERDDDGLPKKWPVDASYG
ATR 300 FISSSVIITTTCHSDGSILALALLLFLILLALALLWWFWPLCCTVIIKEVPPPPARESHENKIK

TEM8 385 GRGVGGIKRMEVRWGEKGSTEEGAKLEKAKNARVKMPEQYEPKPRNLNNNMRPSSPRKWYSPIKGKLDALWVLLRKG

TEM8 465 YDRVSVMRQPQGDTGRCINFTRVKNNQPAKYPLNNAYHTSSPPAPAPIYTPPPAPHCFFFFPSAPTPPISSPPSTLPPP

TEM8 545 QAPPPKRAAPPSRPPRPSV

FIG 1

SEQUENCE LISTING

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 Collier, Robert J.
 Mogridge, Jeremy S.

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 Glu Arg Arg Ala Leu Gly Ile Gly Phe Gln Trp Leu Ser Leu Ala Thr
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 Leu Val Leu Ile Cys Ala Gly Gln Gly Arg Arg Glu Asp Gly Gly
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 Gly Ser Val Leu His His Trp Asn Glu Ile Tyr Tyr Phe Val Glu Gln
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Phe Ser Thr Arg Gly Thr Thr Leu Met Lys Leu Thr Glu Asp Arg Glu	
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Gln Ile Arg Gln Gly Leu Glu Leu Gln Lys Val Leu Pro Gly Gly	
105 110 115	
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Asp Thr Tyr Met His Glu Gly Phe Glu Arg Ala Ser Glu Gln Ile Tyr	
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tat gaa aac aga caa ggg tac agg aca gcc agc gtc atc att gct ttg	547
Tyr Glu Asn Arg Gln Gly Tyr Arg Thr Ala Ser Val Ile Ile Ala Leu	
135 140 145	
act gat gga gaa ctc cat gaa gat ctc ttt ttc tat tca gag agg gag	595
Thr Asp Gly Glu Leu His Glu Asp Leu Phe Phe Tyr Ser Glu Arg Glu	
150 155 160	
gct aat agg tct cga gat ctt ggt gca att gtt tac tgt gtt ggt gtg	643
Ala Asn Arg Ser Arg Asp Leu Gly Ala Ile Val Tyr Cys Val Gly Val	
165 170 175 180	
aaa gat ttc aat gag aca cag ctg gcc cg att gcg gac agt aag gat	691
Lys Asp Phe Asn Glu Thr Gln Leu Ala Arg Ile Ala Asp Ser Lys Asp	
185 190 195	
cat gtg ttt ccc gtg aat gac ggc ttt cag gct ctg caa ggc atc atc	739
His Val Phe Pro Val Asn Asp Gly Phe Gln Ala Leu Gln Gly Ile Ile	
200 205 210	
cac tca att ttg aag aag tcc tgc atc gaa att cta gca gct gaa cca	787
His Ser Ile Leu Lys Lys Ser Cys Ile Glu Ile Leu Ala Ala Glu Pro	
215 220 225	
tcc acc ata tgt gca gga gag tca ttt caa gtt gtc gtg aga gga aac	835
Ser Thr Ile Cys Ala Gly Glu Ser Phe Gln Val Val Val Arg Gly Asn	
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Gly Phe Arg His Ala Arg Asn Val Asp Arg Val Leu Cys Ser Phe Lys	
245 250 255 260	
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Ile Asn Asp Ser Val Thr Leu Asn Glu Lys Pro Phe Ser Val Glu Asp	
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Thr Tyr Leu Leu Cys Pro Ala Pro Ile Leu Lys Glu Val Gly Met Lys	
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Ala Ala Leu Gln Val Ser Met Asn Asp Gly Leu Ser Phe Ile Ser Ser	
295 300 305	

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Trp Phe Trp Pro Leu Cys Cys Thr Val Ile Ile Lys Glu Val Pro Pro	
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Pro Pro Ala Glu Glu Ser Glu Glu Asn Lys Ile Lys	
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aaaaaaaaaa aaaaaaaaaa	1414

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<213> Homo sapiens

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Leu Asp Lys Ser Gly Ser Val Leu His His Trp Asn Glu Ile Tyr Tyr	
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Phe Val Glu Gln Leu Ala His Lys Phe Ile Ser Pro Gln Leu Arg Met	
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Ser Phe Ile Val Phe Ser Thr Arg Gly Thr Thr Leu Met Lys Leu Thr	
85 90 95	
Glu Asp Arg Glu Gln Ile Arg Gln Gly Leu Glu Leu Gln Lys Val	
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Leu Pro Gly Gly Asp Thr Tyr Met His Glu Gly Phe Glu Arg Ala Ser	
115 120 125	
Glu Gln Ile Tyr Tyr Glu Asn Arg Gln Gly Tyr Arg Thr Ala Ser Val	
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Ile Ile Ala Leu Thr Asp Gly Glu Leu His Glu Asp Leu Phe Phe Tyr
 145 150 155 160

 Ser Glu Arg Glu Ala Asn Arg Ser Arg Asp Leu Gly Ala Ile Val Tyr
 165 170 175

 Cys Val Gly Val Lys Asp Phe Asn Glu Thr Gln Leu Ala Arg Ile Ala
 180 185 190

 Asp Ser Lys Asp His Val Phe Pro Val Asn Asp Gly Phe Gln Ala Leu
 195 200 205

 Gln Gly Ile Ile His Ser Ile Leu Lys Lys Ser Cys Ile Glu Ile Leu
 210 215 220

 Ala Ala Glu Pro Ser Thr Ile Cys Ala Gly Glu Ser Phe Gln Val Val
 225 230 235 240

 Val Arg Gly Asn Gly Phe Arg His Ala Arg Asn Val Asp Arg Val Leu
 245 250 255

 Cys Ser Phe Lys Ile Asn Asp Ser Val Thr Leu Asn Glu Lys Pro Phe
 260 265 270

 Ser Val Glu Asp Thr Tyr Leu Leu Cys Pro Ala Pro Ile Leu Lys Glu
 275 280 285

 Val Gly Met Lys Ala Ala Leu Gln Val Ser Met Asn Asp Gly Leu Ser
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 Phe Ile Ser Ser Val Ile Ile Thr Thr Thr His Cys Ser Asp Gly
 305 310 315 320

 Ser Ile Leu Ala Ile Ala Leu Leu Ile Leu Phe Leu Leu Ala Leu
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 factor A domain consensus sequence

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 35 40 45

Ser Asp Ala Arg Val Leu Phe Pro Leu Asn Asp Ser Gln Ser Lys Asp
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Ala Leu Leu Glu Ala Leu Ala Asn Leu Ser Tyr Ser Leu Gly Gly
 65 70 75 80

Thr Asn Leu Gly Ala Ala Leu Glu Tyr Ala Leu Glu Asn Leu Phe Ser
 85 90 95

Glu Ser Ala Gly Ser Arg Arg Gly Ala Pro Lys Val Leu Ile Leu Ile
 100 105 110

Thr Asp Gly Glu Ser Asn Asp Gly Gly Glu Asp Ile Leu Lys Ala Ala
 115 120 125

Lys Glu Leu Lys Arg Ser Gly Val Lys Val Phe Val Val Gly Val Gly
 130 135 140

Asn Ala Val Asp Glu Glu Leu Lys Lys Leu Ala Ser Ala Pro Gly
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Gly Val Phe Ala Val Glu Asp Leu Pro Glu Leu Leu Asp Leu Leu Ile
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Asp Leu Leu Leu
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Gly Leu Asp Ile Gly Pro Thr Lys Thr Gln Val Gly Leu Ile Gln Tyr
 35 40 45

Ala Asn Asn Pro Arg Val Val Phe Asn Leu Asn Thr Tyr Lys Thr Lys
 50 55 60

Glu Glu Met Ile Val Ala Thr Ser Gln Thr Ser Gln Tyr Gly Gly Asp
 65 70 75 80

Leu Thr Asn Thr Phe Gly Ala Ile Gln Tyr Ala Arg Lys Tyr Ala Tyr
 85 90 95

Ser Ala Ser Gly Gly Arg Arg Ser Ala Ala Thr Lys Val Met Val Val
 100 105 110

Val Thr Asp Gly Glu Ser His Asp Gly Ser Met Leu Lys Ala Val Ile
 115 120 125

Asp Gln Cys Asn His Asp Asn Ile Leu Arg Phe Gly Ile Ala Val Leu
 130 135 140

Gly Tyr Leu Asn Arg Asn Ala Leu Asp Thr Lys Asn Leu Ile Lys Glu
 145 150 155 160

Ile Lys Ala Ile Ala Ser Ile Pro Thr Glu Arg Tyr Phe Phe Asn Val
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Ile Phe Ser Ile Glu Gly
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ctgtctctccc cgggctgcgg gcc atg gcc acg gcg gag cgg aga gcc ctc ggc 173

Met Ala Thr Ala Glu Arg Arg Ala Leu Gly

1 5 10

atc ggc ttc cag tgg ctc tct ttg gcc act ctg gtg ctc atc tgc gcc 221
 Ile Gly Phe Gln Trp Leu Ser Leu Ala Thr Leu Val Leu Ile Cys Ala

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 Gly Gln Gly Gly Arg Arg Glu Asp Gly Gly Pro Ala Cys Tyr Gly Gly

30 35 40

ttt gac ctg tac ttc att ttg gac aaa tca gga agt gtg ctg cac cac 317
 Phe Asp Leu Tyr Phe Ile Leu Asp Lys Ser Gly Ser Val Leu His His

45 50 55

tgg aat gaa atc tat tac ttt gtg gaa cag ttg gct cac aaa ttc atc Trp Asn Glu Ile Tyr Tyr Phe Val Glu Gln Leu Ala His Lys Phe Ile 60 65 70	365
agc cca cag ttg aga atg tcc ttt att gtt ttc tcc acc cga gga aca Ser Pro Gln Leu Arg Met Ser Phe Ile Val Phe Ser Thr Arg Gly Thr 75 80 85 90	413
acc tta atg aaa ctg aca gaa gac aga gaa caa atc cgt caa ggc cta Thr Leu Met Lys Leu Thr Glu Asp Arg Glu Gln Ile Arg Gln Gly Leu 95 100 105	461
gaa gaa ctc cag aaa gtt ctg cca gga gga gac act tac atg cat gaa Glu Glu Leu Gln Lys Val Leu Pro Gly Gly Asp Thr Tyr Met His Glu 110 115 120	509
gga ttt gaa agg gcc agt gag cag att tat tat gaa aac aga caa ggg Gly Phe Glu Arg Ala Ser Glu Gln Ile Tyr Tyr Glu Asn Arg Gln Gly 125 130 135	557
tac agg aca gcc agc gtc atc att gct ttg act gat gga gaa ctc cat Tyr Arg Thr Ala Ser Val Ile Ile Ala Leu Thr Asp Gly Glu Leu His 140 145 150	605
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Ala Pro Ile Leu Lys Glu Val Gly Met Lys Ala Ala Leu Gln Val Ser	
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atg aac gat ggc ctc tct ttt atc tcc agt tct gtc atc atc acc acc	1085
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Thr His Cys Ser Asp Gly Ser Ile Leu Ala Ile Ala Leu Leu Ile Leu	
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Phe Leu Leu Leu Ala Leu Ala Leu Leu Trp Trp Phe Trp Pro Leu Cys	
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Cys Thr Val Ile Ile Lys Glu Val Pro Pro Pro Pro Ala Glu Glu Ser	
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 65 70 75 80
 Ser Phe Ile Val Phe Ser Thr Arg Gly Thr Thr Leu Met Lys Leu Thr
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Glu Gln Ile Tyr Tyr Glu Asn Arg Gln Gly Tyr Arg Thr Ala Ser Val
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 Ile Ile Ala Leu Thr Asp Gly Glu Leu His Glu Asp Leu Phe Phe Tyr
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 Ser Glu Arg Glu Ala Asn Arg Ser Arg Asp Leu Gly Ala Ile Val Tyr
 165 170 175
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 180 185 190
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 Gln Gly Ile Ile His Ser Ile Leu Lys Lys Ser Cys Ile Glu Ile Leu
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 Ala Ala Glu Pro Ser Thr Ile Cys Ala Gly Glu Ser Phe Gln Val Val
 225 230 235 240
 Val Arg Gly Asn Gly Phe Arg His Ala Arg Asn Val Asp Arg Val Leu
 245 250 255
 Cys Ser Phe Lys Ile Asn Asp Ser Val Thr Leu Asn Glu Lys Pro Phe
 260 265 270
 Ser Val Glu Asp Thr Tyr Leu Leu Cys Pro Ala Pro Ile Leu Lys Glu
 275 280 285
 Val Gly Met Lys Ala Ala Leu Gln Val Ser Met Asn Asp Gly Leu Ser
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 Phe Ile Ser Ser Ser Val Ile Ile Thr Thr Thr His Cys Ser Asp Gly
 305 310 315 320
 Ser Ile Leu Ala Ile Ala Leu Ile Leu Ile Phe Leu Leu Leu Ala Leu
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 Ala Leu Leu Trp Trp Phe Trp Pro Leu Cys Cys Thr Val Ile Ile Lys
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 Glu Val Pro Pro Pro Ala Glu Glu Ser Glu Glu Asp Asp Asp
 355 360 365
 Gly Leu Pro Lys Lys Trp Pro Thr Val Asp Ala Ser Tyr Tyr Gly
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 Gly Arg Gly Val Gly Gly Ile Lys Arg Met Glu Val Arg Trp Gly Glu
 385 390 395 400
 Lys Gly Ser Thr Glu Glu Gly Ala Lys Leu Glu Lys Ala Lys Asn Ala
 405 410 415
 Arg Val Lys Met Pro Glu Gln Glu Tyr Glu Phe Pro Glu Pro Arg Asn
 420 425 430

Leu Asn Asn Asn Met Arg Arg Pro Ser Ser Pro Arg Lys Trp Tyr Ser
 435 440 445
 Pro Ile Lys Gly Lys Leu Asp Ala Leu Trp Val Leu Leu Arg Lys Gly
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 Tyr Asp Arg Val Ser Val Met Arg Pro Gln Pro Gly Asp Thr Gly Arg
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 485 490 495
 Leu Asn Asn Ala Tyr His Thr Ser Ser Pro Pro Pro Ala Pro Ile Tyr
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 Thr Pro Pro Pro Pro Ala Pro His Cys Pro Pro Pro Pro Pro Ser Ala
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 Ala Thr Leu Val Leu Ile Cys Ala Gly Gln Gly Arg Arg Glu Asp
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Ile Val Phe Ser Thr Arg Gly Thr Thr Leu Met Lys Leu Thr Glu Asp	
85 90 95	
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Gly Gly Asp Thr Tyr Met His Glu Gly Phe Glu Arg Ala Ser Glu Gln	
115 120 125 130	
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Ile Tyr Tyr Glu Asn Arg Gln Gly Tyr Arg Thr Ala Ser Val Ile Ile	
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agg gag gct aat agg tct cga gat ctt ggt gca att gtt tac tgt gtt	646
Arg Glu Ala Asn Arg Ser Arg Asp Leu Gly Ala Ile Val Tyr Cys Val	
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Gly Val Lys Asp Phe Asn Glu Thr Gln Leu Ala Arg Ile Ala Asp Ser	
180 185 190	
aag gat cat gtg ttt ccc gtg aat gac ggc ttt cag gct ctg caa ggc	742
Lys Asp His Val Phe Pro Val Asn Asp Gly Phe Gln Ala Leu Gln Gly	
195 200 205 210	
atc atc cac tca att ttg aag aag tcc tgc atc gaa att cta gca gct	790
Ile Ile His Ser Ile Leu Lys Ser Cys Ile Glu Ile Leu Ala Ala	
215 220 225	
gaa cca tcc acc ata tgt gca gga gag tca ttt caa gtt gtc gtg aga	838
Glu Pro Ser Thr Ile Cys Ala Gly Glu Ser Phe Gln Val Val Val Arg	
230 235 240	
gga aac ggc ttc cga cat gcc cgc aac gtg gac agg gtc ctc tgc agc	886
Gly Asn Gly Phe Arg His Ala Arg Asn Val Asp Arg Val Leu Cys Ser	
245 250 255	
ttc aag atc aat gac tcg gtc aca ctc aat gag aag ccc ttt tct gtg	934
Phe Lys Ile Asn Asp Ser Val Thr Leu Asn Glu Lys Pro Phe Ser Val	
260 265 270	

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<213> *Homo sapiens*

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Met Ala Thr Ala Glu Arg Arg Ala Leu Gly Ile Gly Phe Gln Trp Leu
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Ser Leu Ala Thr Leu Val Leu Ile Cys Ala Gly Gln Gly Gly Arg Arg
 20 25 30

Glu Asp Gly Gly Pro Ala Cys Tyr Gly Gly Phe Asp Leu Tyr Phe Ile
 35 40 45

Leu Asp Lys Ser Gly Ser Val Leu His His Trp Asn Glu Ile Tyr Tyr
 50 55 60

Phe Val Glu Gln Leu Ala His Lys Phe Ile Ser Pro Gln Leu Arg Met
 65 70 75 80

Ser Phe Ile Val Phe Ser Thr Arg Gly Thr Thr Leu Met Lys Leu Thr
 85 90 95

Glu Asp Arg Glu Gln Ile Arg Gln Gly Leu Glu Glu Leu Gln Lys Val
 100 105 110

Leu Pro Gly Gly Asp Thr Tyr Met His Glu Gly Phe Glu Arg Ala Ser
 115 120 125

Glu Gln Ile Tyr Tyr Glu Asn Arg Gln Gly Tyr Arg Thr Ala Ser Val
 130 135 140

Ile Ile Ala Leu Thr Asp Gly Glu Leu His Glu Asp Leu Phe Phe Tyr
 145 150 155 160

Ser Glu Arg Glu Ala Asn Arg Ser Arg Asp Leu Gly Ala Ile Val Tyr
 165 170 175

Cys Val Gly Val Lys Asp Phe Asn Glu Thr Gln Leu Ala Arg Ile Ala
 180 185 190

Asp Ser Lys Asp His Val Phe Pro Val Asn Asp Gly Phe Gln Ala Leu
 195 200 205

Gln Gly Ile Ile His Ser Ile Leu Lys Lys Ser Cys Ile Glu Ile Leu
 210 215 220

Ala Ala Glu Pro Ser Thr Ile Cys Ala Gly Glu Ser Phe Gln Val Val
 225 230 235 240

Val Arg Gly Asn Gly Phe Arg His Ala Arg Asn Val Asp Arg Val Leu
 245 250 255

Cys Ser Phe Lys Ile Asn Asp Ser Val Thr Leu Asn Glu Lys Pro Phe
 260 265 270

Ser Val Glu Asp Thr Tyr Leu Leu Cys Pro Ala Pro Ile Leu Lys Glu
 275 280 285

Val Gly Met Lys Ala Ala Leu Gln Val Ser Met Asn Asp Gly Leu Ser
 290 295 300

Phe Ile Ser Ser Ser Val Ile Ile Thr Thr Thr His Cys Ser Leu His
 305 310 315 320

Lys Ile Ala Ser Gly Pro Thr Thr Ala Ala Cys Met Glu
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 ctgctctccc cgggctgccc gccatggcca cggcggagcga gagagccctc ggcacatcggt 180
 tccagtggct ctcacggcca ctctgggtct catctgcgcc gggcaagggg gacgcaggga 240
 ggatgggggtt ccagcctgct acggcggatt tgacctgtac ttcattttgg acaaattcagg 300
 aagtgtgctg caccactgga atgaaatcta ttactttgtg gaacagttgg ctcacaaatt 360
 catcagccca cagttgaga atg tcc ttt att gtt ttc tcc acc cga gga aca 412
 Met Ser Phe Ile Val Phe Ser Thr Arg Gly Thr
 1 5 10

acc tta atg aaa ctg aca gaa gac aga gaa caa atc cgt caa ggc cta 460
 Thr Leu Met Lys Leu Thr Glu Asp Arg Glu Gln Ile Arg Gln Gly Leu
 15 20 25

gaa gaa ctc cag aaa gtt ctg cca gga gga gac act tac atg cat gaa 508
 Glu Glu Leu Gln Lys Val Leu Pro Gly Gly Asp Thr Tyr Met His Glu
 30 35 40

gga ttt gaa agg gcc agt gag cag att tat tat gaa aac aca gaa ggg 556
 Gly Phe Glu Arg Ala Ser Glu Gln Ile Tyr Tyr Glu Asn Arg Gln Gly
 45 50 55

tac agg aca gct agc gtc atc att gct ttg act gat gga gaa ctc cat 604
 Tyr Arg Thr Ala Ser Val Ile Ile Ala Leu Thr Asp Gly Glu Leu His
 60 65 70 75

gaa gat ctc ttt ttc tat tca gag agg gag gct aat agg tct cga gat 652
 Glu Asp Leu Phe Phe Tyr Ser Glu Arg Glu Ala Asn Arg Ser Arg Asp
 80 85 90

ctt ggt gca att gtt tac tgt gtt ggt gtg aaa gat ttc aat gag aca 700
 Leu Gly Ala Ile Val Tyr Cys Val Gly Val Lys Asp Phe Asn Glu Thr
 95 100 105

cag ctg gcc egg att gcg gac agt aag gat cat gtg ttt ccc gtg aat	748		
Gln Leu Ala Arg Ile Ala Asp Ser Lys Asp His Val Phe Pro Val Asn			
110	115	120	
gac ggc ttt cag gct ctg caa ggc atc atc cac tca att ttg aag aag	796		
Asp Gly Phe Gln Ala Leu Gln Gly Ile Ile His Ser Ile Leu Lys Lys			
125	130	135	
tcc tgc atc gaa att cta gca gct gaa cca tcc acc ata tgt gca gga	844		
Ser Cys Ile Glu Ile Leu Ala Ala Glu Pro Ser Thr Ile Cys Ala Gly			
140	145	150	155
gag tca ttt caa gtt gtc gtg aga gga aac ggc ttc cga cat gcc cgc	892		
Glu Ser Phe Gln Val Val Val Arg Gly Asn Gly Phe Arg His Ala Arg			
160	165	170	
aac gtg gac agg gtc ctc tgc agc ttc aag atc aat gac tcg gtc aca	940		
Asn Val Asp Arg Val Leu Cys Ser Phe Lys Ile Asn Asp Ser Val Thr			
175	180	185	
ctc agt aag tcc ttg cag agt cca tgg gtt tct tcg aca agt ggc ttc	988		
Leu Ser Lys Ser Leu Gln Ser Pro Trp Val Ser Ser Thr Ser Gly Phe			
190	195	200	
aag gaa ggg aat tcc cac cct tgt ctt cca gca agg cca cac aca	1033		
Lys Glu Gly Asn Ser His Pro Cys Leu Pro Ala Arg Pro His Thr			
205	210	215	
tgaaaaccagc agaaaagagt cttatgtct ggaaagaccc ccagcaaggg catagtgagc	1093		
ccttacagtg gttcagtca gaaaaggcac cacttgggtg ggcacagccc catgggtgtc	1153		
caacttgta agcagagcaa ggctggactt gagtccccgt cctccacaaa acacagagcc	1213		
acaagccccca gccctgcagc agccctccgg aagcagcggg gcaactggttt cttgtcccc	1273		
tgccatctac cgagtggctc actctcagggt gggagtgctg gtgatggta attaggactg	1333		
cagaaacatg agcctcctta acaaagtatt gggactctta agggtaagtg tgaaaaagga	1393		
atggtctaaa tgcattaatc ttgaataaac cgaaaaccaa acc	1436		

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Met Ser Phe Ile Val Phe Ser Thr Arg Gly Thr Thr Leu Met Lys Leu
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Thr Glu Asp Arg Glu Gln Ile Arg Gln Gly Leu Glu Leu Gln Lys
20 25 30

Val Leu Pro Gly Gly Asp Thr Tyr Met His Glu Gly Phe Glu Arg Ala
35 40 45

Ser Glu Gln Ile Tyr Tyr Glu Asn Arg Gln Gly Tyr Arg Thr Ala Ser
50 55 60

Val Ile Ile Ala Leu Thr Asp Gly Glu Leu His Glu Asp Leu Phe Phe
65 70 75 80

Tyr Ser Glu Arg Glu Ala Asn Arg Ser Arg Asp Leu Gly Ala Ile Val
85 90 95

Tyr Cys Val Gly Val Lys Asp Phe Asn Glu Thr Gln Leu Ala Arg Ile
100 105 110

Ala Asp Ser Lys Asp His Val Phe Pro Val Asn Asp Gly Phe Gln Ala
115 120 125

Leu Gln Gly Ile Ile His Ser Ile Leu Lys Lys Ser Cys Ile Glu Ile
130 135 140

Leu Ala Ala Glu Pro Ser Thr Ile Cys Ala Gly Glu Ser Phe Gln Val
145 150 155 160

Val Val Arg Gly Asn Gly Phe Arg His Ala Arg Asn Val Asp Arg Val
165 170 175

Leu Cys Ser Phe Lys Ile Asn Asp Ser Val Thr Leu Ser Lys Ser Leu
180 185 190

Gln Ser Pro Trp Val Ser Ser Thr Ser Gly Phe Lys Glu Gly Asn Ser
195 200 205

His Pro Cys Leu Pro Ala Arg Pro His Thr
210 215